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The ability of the soil extract to methylate mercury is influenced by temperature, mercuric ion concentration, and solution pH. The methylating ability of the soil extract was stable at high temperatures (121° C), but was lost after exposure to ultraviolet radiation.

When the 0.5N sodium hydroxide extract of soil was separated into a soluble and insoluble fraction, the ability to methylate mercuric ion remained with the soluble fraction. It was found that the methylating factor was lost when the 0.5N sodium hydroxide extract was dialyzed against distilled water. Further work showed that the methylating factor passes through dialysis tubing into the distilled water.

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ABIOLOGICAL METHYLATION OF MERCURY IN SOIL

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ABSTRACT

The results from this work define several factors influencing the methylation of mercuric ion in soil. Two of the most important findings were that it is possible to extract the mercury methylating factor from soil with a solution of 0.5N sodium hydroxide (NaOH) and that this factor is responsible for the abiological methylation of mercury in the soils under investigation.

The ability of the soil extract to methylate mercury is influenced by temperature, mercuric ion concentration, and solution pH. In addition, it was found that the methylating ability of the soil extract was stable at high temperatures (121° C), but was lost after exposure to ultraviolet radiation.

When the 0.5N NaOH extract of soil was separated into a soluble fraction and an insoluble precipitate, the ability to methylate mercuric ion remained with the soluble fraction. It was found that the methylating factor was lost when the 0.5N NaOH extract was dialyzed against distilled water. Other work showed that the methylating factor passes through dialysis tubing into the distilled water.

ACKNOWLEDGMENTS

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INTRODUCTION

In an earlier work (Rogers, 1975), it was shown that mercuric ion is methylated under a variety of conditions in alkaline, agriculture soils. That study confirmed the work of Beckert et al. (1974) who found methylmercury in desert soils which had been amended with mercuric nitrate. In neither study was the mechanism for the methylation of mercury discovered. However, it was suggested that there was a possibility for an abiotic process (Rogers, 1975).

Since it has been established that mercuric ion can be abiotically methylated by a variety of substances (Bertilsson and Neujahr, 1971; Imura et al., 1971; DeSimone, 1972; Brinckman, Iverson, and Blair, 1975), an abiotic pathway seemed probable. A partial confirmation of the necessity of soil organic matter for the abiotic methylation of mercury in soil came as a result of two preliminary experiments. A silty clay loam soil which had been used in a previous study (Rogers, 1975) was ignited at 600° C for 24 hours. The ashed soil was then amended with mercuric ion at the rate of 25,000 micrograms of mercury (μg Hg) per 50 grams (g) of ashed soil. After one week of incubation, these soils contained no methylmercury, while an unashed control soil did contain significant quantities of methylmercury. Soil was also exposed to a process of wet oxidation. Such a treatment can sterilize soil. However, only after several repeated oxidation steps was it possible to destroy the methylating ability of the soil, which suggested that methylation was not biological and that the methylating factor was associated with soil organic material.

While attempting to extract organic material from the above soil, it was discovered that a 0.5N NaOH extract of the soils was capable of methylating introduced mercuric ions. This report is the results of studies involving mercury reactions with NaOH extracts of soil.

CONCLUSIONS

The following conclusions were drawn from the current study:

1. A constituent can be extracted from soil which will methylate mercuric ion abiologically.

2. The methylating substance is associated with the lower molecular weight fraction of the soil organic matter.

3. Soil extracts exposed to ultraviolet (UV) radiation lose the ability to methylate mercury.

4. The rate of conversion of mercury into methylmercury is dependent upon mercuric ion concentration, solution pH, and temperature.

RECOMMENDATIONS FOR FUTURE RESEARCH

Further experimental refinements and identification of the compound responsible for the methylation of mercury in sodium hydroxide extracts of soils are required. Once identified, it will be possible to analyze other soils and even aquatic systems for the compound. With those findings it should be possible to identify areas where significant methylation of mercury is likely to occur.

MATERIALS AND METHODS

Agricultural soils used for this investigation were obtained from the area in southeastern Nevada. The soils were of three different textural types. These were a loamy sand, fine sandy loam, and silty clay loam. For convenience these soils will be referred to as sand, loam, and clay. The soils contained 0.53%, 1.30%, and 3.44% organic carbon, respectively. A more complete description of the soils and their processing is found elsewhere (Rogers, 1975).

Extraction of organic material from the soils was done by Stevenson's (1965) method for the extraction of soil humus with two modifications. The soils were not acid-washed prior to extraction and 50 grams (g) of soil were used instead of 40 g. The resulting sodium hydroxide (NaOH) extracts had the following optical densities at pH 13: sand, 0.07; loam, 0.18; and clay, 0.56. The solutions resulting from the extraction process were stored in amber glass bottles.

Unless noted otherwise, the 0.5N NaOH extract obtained from these soils was used for each phase of this study. The extracts were amended with mercuric nitrate [$Hg(NO_3)_2$] at the rate of 25,000 micrograms (µg) Hg as $Hg(NO_3)_2$ per 50 milliliters (ml) of extract (500 ppm Hg). The extracts were prepared for amendment with mercuric nitrate as follows. Before mercury was added, the extract pH was decreased below 7 by adding concentrated nitric acid (HNO_3). After the pH of the extract had been adjusted, 2 ml of a solution containing 12,500 ppm Hg were added to 50 ml of extract. The pH of the amended extract was then adjusted to the desired pH with further additions of either concentrated HNO_3 or 20% NaOH while the extract was being stirred with a magnetic stirrer. Unless noted otherwise, there were four replications per treatment of the mercury-extract solutions. These solutions were incubated at 23° C on a shaker.

After incubation, methylmercury was extracted from these solutions using a modified Westoo method (1966) discussed in a previous study (Rogers, 1975). With the 0.5N NaOH extracts, however, the solution pH was lowered to 1 with concentrated hydrochloric acid (HCl). Benzene which was used to extract the methylmercury was analyzed as previously described (Rogers, 1975) using gas chromatography and a nickel-63 linear electron capture detector. For additional confirmation of methylmercury, a random sample of the extracts was submitted to analysis by a gas chromatograph equipped with an element-specific microwave emission spectrometric detector systems (Talmi, 1975).

In order to evaluate the effectiveness of the extraction procedure, standard solutions consisting of 1 µg Hg as methylmercury chloride (CH_3HgCl)

in distilled water were added to the 0.5N NaOH extract of the three soils. These solutions were extracted using the above method and the quantity of the extracted methylmercury was compared to the amount initially added. Using this method it was determined that the extract from the sand had a 66% recovery of mercury as methylmercury, loam extract, 49%, and clay extract, 53%. In addition, no methylmercury was founded in zero time extractions of $Hg(NO_3)_2$ amended NaOH extracts of the three soils.

Either one-way analysis of variance (ANOV) or polynomial regression was used for the statistical testing of the data. When ANOV indicated a significant difference between treatments, an orthogonal comparison was then used to identify differences between individual treatment means.

RESULTS

EFFECT OF pH

The solubility of mercury in solution, especially at the concentration used, is mediated by solution pH. For a solution extract of soils, pH not only affects the solubility of mercury, but also the sorption of mercury by organic complexes. It was assumed that the availability of mercuric ion would increase as the soil extract became acidic. If this were true, the methylation of mercury should be enhanced by the increased availability of mercuric ions. An experiment was initiated to determine the methylating ability of the 0.5N NaOH extracts of the three soils at various pH's. Extracts of each of the soils were adjusted to pH 2.0, 4.5, 5.5, 7.5, and 9.0 with concentrated HNO_3. After the extracts had been amended with 25,000 µg Hg as $Hg(NO_3)_2$, the solution pH was checked and any necessary corrections were made. The amended solutions were then shaken at 25° C for 1 day.

Data from this study show the effect pH has on the production of methylmercury in these extracts (Figure 1). In all extracts there was a decrease in the occurrence of methylmercury when the solution pH was above 4.5. There was a continued decrease in methylmercury with increasing pH in both the clay and loam extracts with the least amount of methylmercury being obtained at pH 9.0. The sand extract remained static from pH 5.5 through the end point. An anomaly occurred with the clay extract at pH 2.0. There was a lower concentration of methylmercury at this pH than at pH 4.5. It was noted that these solutions contained a considerable amount of organic precipitate (humic acid). It is probably that complexing of the humic acid rendered some of the mercury or methylation factors unavailable for reaction. Because of the possible problem of complexing the mercury and the relative ease of adjusting and maintaining the soil extracts at pH 4.5, this pH was selected for the other studies. It should be noted that controls containing everything except soil extract (but including 50 ml of 0.5N NaOH) failed to produce methylmercury.

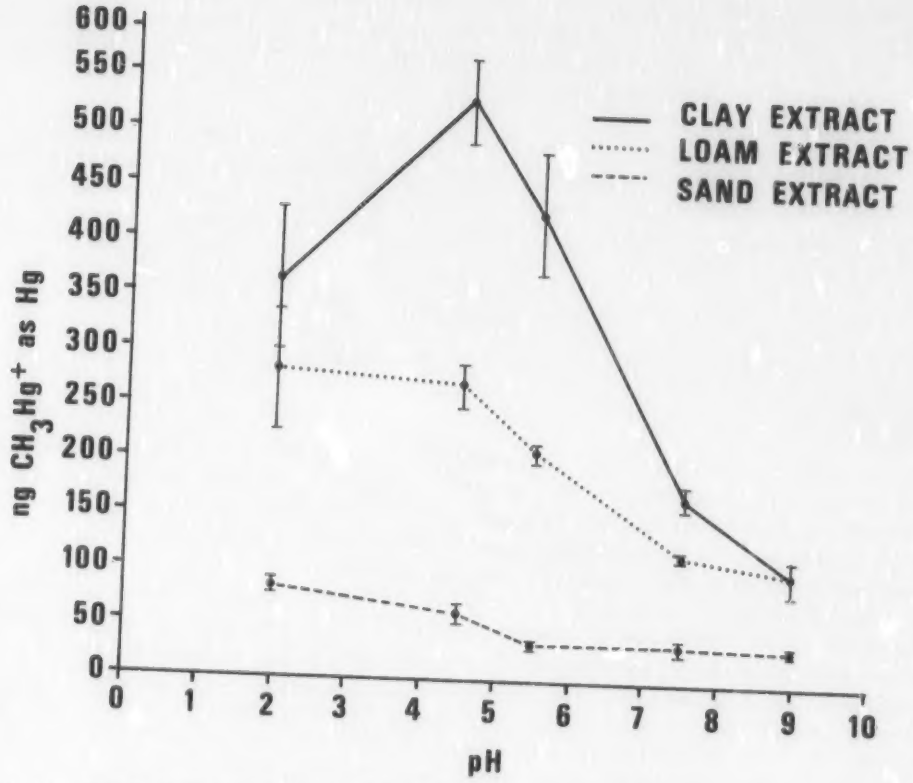

Figure 1. Methylmercury in 0.5N NaOH extracts of soil at various pH values

INCUBATION TIME

The extent of mercury methylation by the three soil extracts was determined for 1, 3, and 7 days of incubation (Table 1). The loam and sand extracts had significant (at the 1% level) increases in methylmercury content between days 1 and 3. The content of methylmercury in the loam extract did not change significantly from day 3 to day 7, while the sand extract decreased in methylmercury by day 7. Analysis of the clay extract revealed no significant increase in methylmercury occurrence between days 1 and 3, but a significant increase (at the 1% level) between days 3 and 7.

The conclusions to be drawn from these data are that the loam and sand extracts reach a maximum methylmercury content within a relatively short period of time. The clay extract requires a longer period of time before significant increases in methylmercury above initial quantities are observed. These data also indicate that methylmercury formation in different soils is not a uniform process.

TABLE 1. METHYLMERCURY OCCURRENCE IN 0.5N NaOH EXTRACT INCUBATED 1, 3, AND 7 DAYS AT 25° C

Extract	Concentration (ng CH_3Hg^+/50 ml Extract) 1 Day	3 Days	7 Days
Clay	622.6	646.3	803.0*
Loam	196.0	299.7*	268.8
Sand	65.5	204.9*	144.8*

* Indicates significance at the 1% level between the same extract with increasing time.

INCUBATION TEMPERATURE

Extracts of the soils were amended with mercuric ions and then shaken at 5° C, 25° C, and 40° C for 1 day. All amended extracts increased in methylmercury content with increased temperature (Figure 2). Regression coefficients for this plot are 13.9, 5.1, and 2.2, for the clay, loam, and sand extracts, respectively, with a significance of 1% for all (F values of 84.9, 92.6, and 38.9; 1, 10 df). From these data it can be seen that the production of methylmercury in the clay extract is substantially elevated.

MERCURY CONCENTRATION

Extracts of the soils were amended with 12,500 µg ng, 25,000 µg Hg, and 37,500 µg Hg as $HgNO_3$ per 50 ml extract. These solutions were then incubated for 1 day.

Figure 3 shows the effect of increased mercury concentration extracts. Regression coefficients for these data are 22.3, 7.6, and 4.9 for the clay, loam, and sand extracts, respectively. Both the clay and loam extracts are significant at the 1% level while the sand extract was not significant at the 5% level (F values of 321.5, 22.1, and 4.9; 1, 10 df). The lack of significance of the sand data was because of a high variability between treatment replications. The clay extract had a good response to increased mercury concentration and this response clearly indicates that the clay extract could methylate mercury under even greater mercury concentrations. The data for the loam and sand extracts indicate that these extracts may have had their methylation factor saturated at the 25,000 µg and 37,500 µg Hg levels.

Fig 2 METHYLMERCURY OCCURRENCE IN 0.5N NaOH EXTRACTS INCUBATED AT VARIOUS TEMPERATURES FOR ONE DAY

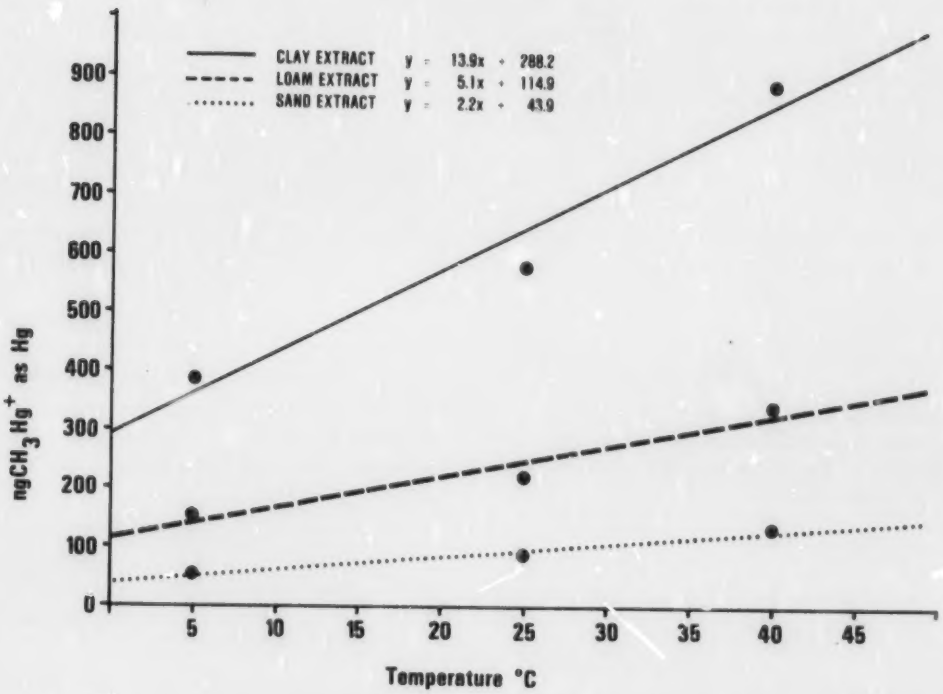

Figure 2. Methylmercury occurrence in 0.5N NaOH extract incubated at various temperatures for 1 day

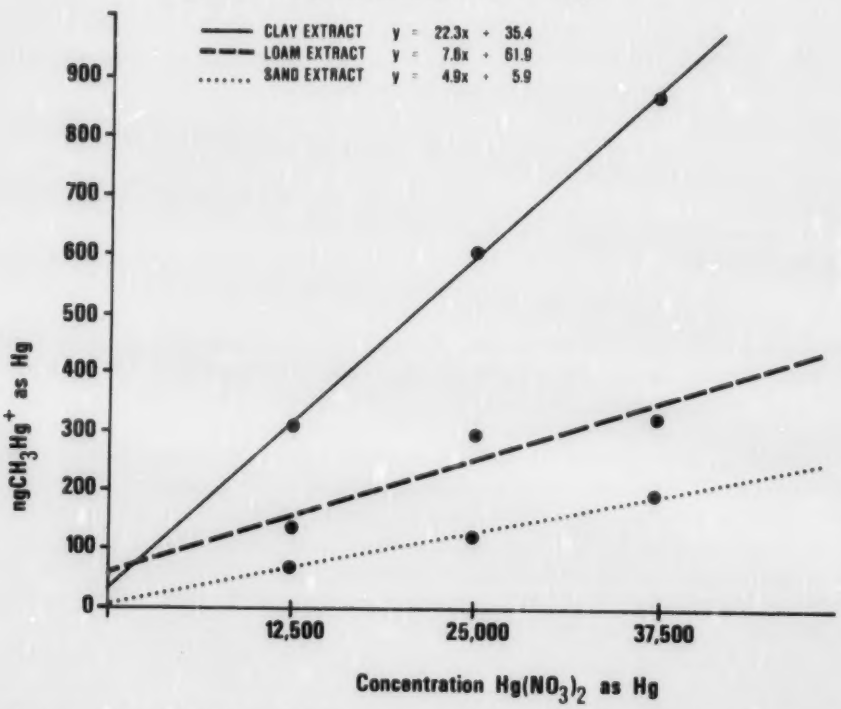

Figure 3. Methylmercury occurrence in 0.5N NaOH extract incubated for 1 day with amendments of varying mercuric nitrate concentrations

CHARACTERISTICS OF THE METHYLATING FACTOR

The mechanism for mercury methylation in these soil extracts is nonbiological. Four petri plates containing 1 ml of extract and 15 ml glucose nitrient agar showed no microbial growth after 1 week of incubation. In a similar experiment, extracts containing 25,000 µg Hg as $Hg(NO_3)_2$ had no microbial contamination after 1 week of solution incubation. Apparently the combination of high Na and Hg concentration did not allow for microbial growth. In addition, quantities of each of the extracts were autoclaved at 121° C and 15 psi for 30 minutes. These sterile extracts were then amended with mercury at the standard rate of 25,000 µg Hg as $Hg(NO_3)_2$ per 50 ml extract and incubated 1 day. Methylmercury was found in all the amended solutions (Table 2). These values showed the same trend as those of non-autoclaved solutions (Table 1).

TABLE 2. METHYLMERCURY OCCURRENCE IN AUTOCLAVED 0.5N NaOH EXTRACT INCUBATED 1 DAY

Extract	Concentration (ng CH_3Hg+/50 ml Extract)
Clay	551.3* ± 29.6**
Loam	296.9 ± 26.5
Sand	121.0 ± 19.7

* Mean of four replications.
** ± standard deviation.

Alkaline extracts of soil are routinely separated by acidification into two different fractions (Stevenson, 1965). The precipitated fraction is designated as humic acids, and the soluble fraction is known as fulvic acid. A study was initiated to determine which of these fractions was responsible for the methylation. The humic acid fraction of the soil extracts was removed. The fulvic acid fraction of each soil was then amended with 25,000 µg Hg as $Hg(NO_3)_2$ per 50 ml extract and was incubated for 1 day. Analysis of these solutions showed that mercuric ions were methylated at approximately the same rate as prior to fraction separation (Table 3). These data suggest that the methylation process is mediated by some of the low-molecular-weight material which composes the fulvic acid fraction.

The corollary to the fulvic acid study was also performed. Samples of four commercially available humic acid precipitations were obtained from Mr. George Baughman of the EPA's laboratory in Athens, Georgia. These materials were designated: Aldrich humic acid, technical grade; Humussauve 7821; Fluka Ag, Buchssg; and Humussauve Naturiumsatz. Sufficient amounts of each of these humic acids were dissolved in 0.5N NaOH so that the resulting mixtures had the same optical density at pH 13 as the clay extract at the same pH. These solutions were then amended with mercury at the rate of 25,000 µg

Hg as $Hg(NO_3)_2$ per 50 ml solution. After 1 day of incubation at pH 4.5 none of the solutions contained methylmercury. More concentrated solutions of the humic materials also failed to produce methylmercury. It was therefore concluded that the methylating factor was not associated with the higher molecular weight humic acid fraction.

TABLE 3. METHYLMERCURY OCCURRENCE IN 0.5N NaOH EXTRACT AND 0.05N NaOH EXTRACT WITH HUMIC ACID REMOVED

	CH_3Hg^+ Detected (ng/50 ml Extract)	
Extract	Complete Extract	Humic Acid Removed
Clay	515.0	427.3**
Loam	172.4	137.2*
Sand	76.6	75.7

* or ** Indicates significance at the 5% or 1% level between the same extract with and without humic acid.

In order to aid in the long-term storage, molecular analysis, and concentration studies, quantities of the 0.5N NaOH extracts from the clay, loam, and sand were freeze dried. The method for drying (Stevenson, 1965) involved the dialysis of the solutions to remove the excess NaOH. Three 500-ml quantities of each solution were dialyzed for 3 days against 30 liters of moving distilled water. The water was changed daily and maintained at a temperature of 50° C. At the end of this period of dialysis the solutions were tested to determine their methylating potential. Incubation of these dialyzed solutions with mercury failed to produce methylmercury. Subsequent dialyzed solutions also failed to produce methylmercury. At first, it was thought that the NaOH was required in the methylation process. To test this hypothesis, NaOH was added to the dialyzed soil extracts so that the resulting solutions contained the same NaOH concentration as before dialysis. Incubation of these soil solutions with mercury also failed to produce methylmercury.

It has been shown that low-molecular-weight components of fulvic acid are lost through dialysis tubing (Mortensen and Himes, 1964; Stevenson and Butler, 1969). Knowing this, an effort was then made to determine if the methylating factor had indeed permeated the dialysis membrane, which according to the manufacturer, Union Carbide, would pass molecules with molecular weights of up to 12,000. In this study, 500 ml of the 0.5N NaOH clay extract was placed into the dialysis tubing. The full tube was suspended in a graduated cylinder and the cylinder was then filled with 500 ml of distilled water. The complete apparatus was then placed in an oven and maintained at 50° C for 24 hours. At the end of this time it was noted that the distilled water had acquired a straw

yellow color. This solution was then amended with 25,000 µg Hg as $Hg(NO_3)_2$ per 50 ml and incubated for 1 day. Upon analysis the solution was found to contain 303 ng CH_3Hg^+ per 50 ml solution and thus confirmed that the methylating factor was lost during dialysis, but remained active when separated from the higher molecular weight soil organics.

The photosensitivity of the methylating factor was determined by exposing samples of the 0.5N NaOH extract of clay to ultraviolet (UV) radiation. A quartz immersion well was used in conjunction with a small, medium-pressure mercury lamp. With 50 ml of the clay extract in the water-cooled immersion well, the UV source was turned on for 16 hours. At the end of this time the extracted solution was bleached and appeared water clear. This solution was amended with 25,000 µg Hg as $Hg(NO_3)_2$ and incubated for 1 day. No methylmercury was found in the mixture at the end of this time. The experiment was repeated and the same results were obtained.

DISCUSSION

The results from this work define several as yet unknown factors influencing the methylation of mercuric ion in soil. Two of the most important findings are that the methylation reaction is abiological and that it is possible to extract the methylating factor from soil. The occurrence of methylmercury was confirmed by both an electron capture detector and an element-specific microwave emission spectrometer detector system (Talmi, 1975).

It had previously been found that mercury could be methylated by non-biological processes. Bertilsson and Neujahn (1971) and Imura et al. (1971) showed that methylcobalamin was capable of transferring methyl groups to mercuric ions. DeSimone (1972) was able to demonstrate the methylation of mercury by trimethylsilyl salts. It is also known that there can be a transfer of methyl groups from other methylated metals to mercuric ion (Brinckman, Iverson, and Blair, 1975). So, the occurrence of an abiotic mechanism for the methylation of mercury in active environmental systems should not be surprising.

While the substance in the soil extracts responsible for the methylation of mercury is not known, the reaction can be defined by the following factors. The reaction produces significant amounts of methylmercury within a 24-hour period (Table 1). Increasing temperature increased the rate of reaction (Figure 2), and increasing concentration of mercury substrate increased the amount of resulting methylmercury (Figure 3). Some variability was noted with the loam and sand extracts when amended with various concentrations of mercury. The production of methylmercury in these solutions is also dependent upon solution pH. The greatest amount of methylmercury was found in those solutions maintained below pH 5.5. The lower pH is thought to allow for a greater availability of the mercury ion.

Through this study it has been possible to delineate several facts about the methylating substance. It was evident from all of the studies that the methylating substance is more available in the clay followed by the loam and then the sand extracts. These results are consistent with data on the

occurrence of methylmercury in the complete soils (Rogers, 1975). The methylating factor can be destroyed by photo-oxidation, thus indicating that it is an organic compound. Temperatures of at least 121° C have no effect on the methylating ability of the soil solutions (Table 2). The molecular weight of the substance is apparently within the range of that material commonly found in the fulvic acid fraction of the soil. This is known because fulvic acid preparations of the soils methylate mercury at the same rate as solutions containing both fulvic and humic acids (Table 3). Also, solutions containing only humic acid were not capable of mercury methylation. And lastly, since the methylating factor passed into distilled water via dialysis tubing it should be possible to further define it.

It is hoped that this work will add emphasis to the importance of the abiotic methylation of mercury not only in soil environments, but also aquatic environments. It is now possible to have at hand a solution which can be not only chemically modified in many ways, but can also be used as a standard in determining the impact on mercury methylation by many of the inorganic compounds of mercury.

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